-- 67. The chimeric animal according to Claim 13, wherein said chimeric animal is used as a source of bone marrow transplantation. --

-- 68. The cell line according to Claim 10, wherein said cell line is used as a source of tissue for grafts. --

-- 69. The chimeric animal according to Claim 13, wherein said chimeric animal is used as a source of tissue for grafts. --

sub C12 70. The chimeric animal according to Claim ~~13, wherein said chimeric animal is created~~ by ~~means~~ other than a xenograft. --

-- 71. The chimeric animal according to Claim 13, wherein said chimeric animal is created by means other than transplantation of non-totipotent cells.

REMARKS

Applicant acknowledges receipt of the Office Action mailed October 29, 1999. Applicant recognizes the extensive review and discussion the Office has devoted to this application and appreciates the extraordinary level of effort devoted by the Examiner and the Office to the application. As recognized by the Examiner, the application is significant in terms of its public policy implications, as well as its own scientific merits.

Claims 1-55 are pending in this application. By this Amendment, Claims 1, 5-7, 10, 13, 16, 28, 32-34, 53, and 55 are amended, Claims 8, 9, 11, 12, 14, 15, 17-27, 35-37, 49, 51, 52, and 54 are canceled without prejudice, and new Claims 56-71 are added. Applicant respectfully requests that the Examiner reconsider the pending claims, allow the claims in view of the amendments and additional information submitted in this Response.

The application as originally drafted and subsequently amended claimed various permutations of a chimeric embryo. More specifically, claims were drawn to chimeric embryos, and cell lines and animals derived from chimeric embryos. The Office has rejected the claims on five separate grounds:

- First, the Examiner has rejected Claims 1-9, 13-37, 39-48, 52, and 54 under 35 U.S.C. § 101 as being directed to non-statutory subject matter, namely as falling within an exception to statutory subject matter as embracing a human being.
- Second, the Examiner has rejected Claims 1, 2, 5-7, 10-18, 28, 29, 32-34, 38-51, 53, and 54 under 35 U.S.C. § 102(b) as being anticipated by certain references disclosing: the introduction of human hematopoietic cells into sheep and mice *in utero*; mouse primordial germ cells and human fetal and embryo cell lines; and transgenic mice.
- Third, the Examiner has rejected Claims 1, 2, 5-9, 19, 20, 23-29, and 32-48 under 35 U.S.C. § 103(a) as being obvious, in view of references disclosing: sheep/goat chimeras; chimeric sheep/goat pregnancies; chick/quail chimeric embryos; and chimeric mice.
- Fourth, the Examiner has rejected all claims under 35 U.S.C. § 112, first paragraph, based on the assertion that, in spite of the asserted obviousness and anticipation of the invention by no less than nine separate references, the specification would not enable one of ordinary skill in the art to practice the invention, citing unpredictable outcomes in chimera formation, and the lack of fecundity of chimeric animals.

- Fifth, the Examiner has rejected all claims under 35 U.S.C. § 112, second paragraph as being vague and indefinite as to what would be considered a chimeric embryo in terms of viability.

Applicant respectfully submits that the variety, multiplicity, and inconsistency of the various rejections support the allowability of the claims as amended.

The subject matter of the appended claims is made by the intervention of man. The claimed subject matter is not naturally occurring and constitutes patentable subject matter under Section 101. The vast array of references cited by the Examiner establishes the level of background knowledge of persons of ordinary skill in the art. In the context of that knowledge, the specification satisfies the requirements of Section 112. The techniques that are needed to make and use the claimed invention are well within the ordinary level of skill in the art as evidenced by the multiple references identified by the Examiner in the Office Actions. In spite of the comprehensiveness of the art, no one has practiced, taught, or suggested the use of these well known and amply documented techniques to make the claimed invention. The Examiner has recognized the richness of the level of ordinary skill, yet, has identified no reference teaching or suggesting the claimed invention as a whole. Applicant respectfully submits that the claims, as amended, are patentable and respectfully requests that the claims be allowed.

I. <u>The Application Claims Patentable Subject Matter (35 U.S.C. § 101)</u>

Claims 1-9, 13-37, 39-48, 52, and 54 are rejected under 35 U.S.C. § 101 as directed to nonstatutory subject matter. Specifically, the Examiner asserts that the claims are unpatentable because they "embrace a human being." This rejection is respectfully traversed. Claims 10-12, 38, 49-51, 53, and 55 were not rejected as "embracing" a human being.

The Examiner asserts that the PTO has rejected claims that encompass a human being under 35 U.S.C. § 101, and requires that claims drawn to animals be expressly limited to "non-human" animals. Despite the lack of an express exception for human beings in § 101, the Examiner asserts that a claim that encompasses a human being is drawn to non-statutory subject matter. Applicant maintains that the subject matter claimed in the present invention is not a human being and that no statutory authority supports the rejection on these grounds.

The rejection is improper for two reasons: (1) it is not a proper statutory requirement for patentability; and (2) the claimed subject matter is not a human being but, rather, man-made chimeric embryos, chimeric cell lines, and chimeric animals derived or isolated from them. Applicant respectfully submits that the Commissioner has no authority to reject the claims of the present invention--that are explicitly "made by man"--on the grounds that they "embrace a human being." Applicant respectfully submits that the above-amended claims and outlined new claims and following remarks obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

Applicant acknowledges Examiner's argument that the PTO is obliged to follow the intent of Congress when administering the patent laws, and that neither the statute itself, nor its legislative history, contains explicit statements as to whether Section 101 was intended to encompass human beings. Examiner alleges that Applicant is arguing in support of the patentability of subject matter "embracing human beings." This is not the case. The "embracing a human being" classification was coined by the original Examiner in the initial Office Action. Applicant's invention does not "embrace a human being." Applicant respectfully submits that the PTO does not have authority to reject an application as "embracing a human being".

Although no chimeras of the sort Applicant claims — i.e., chimeras constructed of embryonic blastomeres and/or embryonic stem cells—have been reported in the scientific literature, chimeras containing human cells have been reported, and these are clearly not considered to be human. For example, Almeida-Porada, et al., transplanted human hematopoietic stem cells from adult bone marrow adult peripheral blood, or fetal liver, into the bone marrow of 30 sheep in utero. They referred to the resulting animals as "human/sheep chimeric lambs" (Almeida-Porada, G. D., Hoffman, R., Manalo, P., Gianni, A. M., and Zanjani, E. D. (1996). Detection of Human Cells in Human/Sheep Chimeric Lambs with in vitro Human Stroma-forming Potential. *Exp Hematol* **24,** 482-7). Brustle, et al., transplanted fetal human brain cells implanted into the cerebral ventricles of embryonic rats, and generated rats containing human neurons and glia in their brains. They state: "These chimeras provide a unique model to study human neural cell migration and differentiation in a functional nervous system" (Brustle, O., Choudhary, K., Karram, K., Huttner, A., Murray, K., Dubois-Dalcq, M., and McKay, R. D. (1998). Chimeric Brains Generated by Intraventricular Transplantation of Fetal Human Brain Cells into Embryonic Rats. *Nat Biotechnol* **16,** 1040-4). In neither of these studies, both of which were supported by the U.S. Government via the National Institutes of Health, is there any suggestion that these chimeras containing human cells are considered to be or "embrace" human beings. It is not valid to consider organisms containing animal and human cells produced the methods of the present invention as "embracing a human being" and those produced by other methods (e.g., those of Almeida-Porada, et al., 1996 and Brustle, et al., 1998) as not "embracing a human being."

The Examiner sets forth the following arguments, based on her assertion that embodiments of the present invention"embrace a human being".

- The Examiner asserts that a grant of patent rights in the present invention would be inconsistent with the constitutional right to privacy, due to the possible restriction on reproductive choices. The Examiner asserts that the reproductive choices of a human being encompassed by the patent claims, or a human being who arose from a patented embryo, would be subject to the patentee's legal right to exclude others from "making" further patented human beings or embryos.
- The Examiner asserts that a patent could give the patentee a legal right to exclude all others from employing, or "using", the patented human being. Such a situation would be tantamount to involuntary servitude in the Examiner's view and, therefore, prohibited by the Thirteenth Amendment.
- The Examiner asserts that the patentability of the present invention conflicts with 35 U.S.C. § 271(g), providing that it is an act of infringement to import products made abroad using a process patented in the United States. The Examiner reasons that if a human were regarded as a "product", under the patent laws, the "product" of using a patented surgical process would be a surgically altered human being. The Examiner contents that if a U.S. resident were to have surgery abroad (where the surgical procedure was patented in the U.S.) and then re-entered the United States, the human being (the product) would be liable for patent infringement.

Applicant maintains its position that it is not making a human being, or anything that "embraces a human being." Applicant's invention is a chimeric embryo, a chimeric cell line, or a chimeric animal that is developed in the laboratory and made by man. The "right to exclude others

from making the invention" is the right to exclude others from making these embryos as described in the application. As such, Examiner's arguments basing a rejection of the current claims on a violation of the constitutional right to privacy and reproductive choices are unfounded. With regard to the Examiner's argument to deny patentability based on the Thirteenth Amendment, this too assumes that the subject of the claims is a human being -- with civil rights. As noted above, it is not. The subject matter as claimed does not conflict with the prohibition of the Thirteenth Amendment on involuntary servitude. As to Examiner's arguments to deny patentability based on 35 U.S.C. § 271(g), these arguments are also unfounded as Applicant's invention is not a human being, nor does it "embrace a human being".

The only issue is whether or not the claimed invention describes *statutory* subject matter. Nowhere does the statute restrict patentability based upon embracing a human being. To support this requirement, the Examiner relies upon the Commissioner's authority as construed by the U.S. Supreme Court in *Diamond v. Chakrabarty,* 447 U.S. 303 (1980) and interpreted by the Commissioner in Guidelines, 1077 OG 24 (Apr. 21, 1987). Neither provides a basis for the present rejection. There is no *statutory* basis for imposing such a rejection under *Chakrabarty.*

The Court in *Chakrabarty* held that statutory subject matter shall "include anything under the sun that is made by man." (447 U.S. at 309). The claimed subject matter is not naturally occurring. It is not disputed by the Examiner that the claimed subject matter is "made by man." A chimeric embryo, a chimeric cell line, or a chimeric animal, derived or isolated from the chimeric embryo, are each made by man and none is naturally occurring.

The Examiner, recognizing that the claimed subject matter falls squarely within the scope of the Court's holding in *Chakrabarty*, that it is "made by man," injects the additional limitation that, although made by man, the invention cannot "embrace a human being."

There are three reasons why the Examiner's "embraces a human being" rejection is not proper. First, it has no statutory or other legal basis. Second, it contravenes established law defining what a "human being" is and the rights flowing from that status. Third, the rejection is inconsistent with established PTO practice of granting patents for a variety of inventions, many of which would "embrace a human being" equally or more than the claimed invention.

The Federal Circuit recently emphasized in *State Street Bank & Trust Co. v. Signature Financial Group*, 149 F.3d 1368 (Fed. Cir. 1998) that neither courts nor the Patent Office are authorized to embellish the statutory requirements for patentability. In *State Street*, the Federal Circuit confronted the so-called "mathematical algorithm" and "business method" exceptions to patentability. *State Street*, 149 F.3d at 1373, 1375-76:

> The repetitive use of the expansive term "any" in § 101 shows Congress's intent not to place any restrictions on the subject matter for which a patent may be obtained beyond those specifically recited in § 101. Indeed, the Supreme Court has acknowledged that Congress intended § 101 to extend to "anything under the sun that is made by man." *Diamond v. Chakrabarty*, 447 U.S. 303, 309, 100 S.Ct. 2204, 65 L.Ed.2d 144 (1980); *see also Diamond v. Diehr*, 450 U.S. 175, 182, 101 S. Ct. 1048, 67 L.Ed.2d 155 (1981).[3] Thus, it is improper to read limitations into § 101 on the subject matter that may be patented where the legislative history indicates that Congress clearly did not intend such limitations. *See Chakrabarty*, 447 U.S. at 308, 100 S.Ct. 2204 ("We have also cautioned that courts 'should not read into the patent laws limitations and conditions which the legislature has not expressed.' " (citations omitted)).

> [3] The Committee Reports accompanying the 1952 Act inform us that Congress intended statutory subject matter to "include anything under the sun that is made by man." S. Rep. No. 82-1979 at 5 (1952); H.R.Rep. No. 82-1923 at 6 (1952).

Id. at 1373.

As the Federal Circuit has held so clearly in *State Street*, "any" invention "made by man" is patentable subject matter. It is for Congress--not the courts or the PTO--to set forth any limitations on patentable subject matter. Congress has not established any limitation based on subject matter that "embraces a human being." The Commissioner lacks the authority to impose one under Section 101.

Even were it possible to overlook the lack of a statutory basis for the new "human being" exception applied in this case--and it is not--such a standard is vague and hopelessly subjective. The Examiner has not specified how the claimed man-made chimeras "embrace a human being" or what features of a human are critical in doing so. "Chimeric embryos and animals containing human cells" can be considered to "embrace a human being" only in extreme cases and, even then, only in a subjective sense, none of which is the subject of this invention. All of the subject matter of the present claims is drawn to chimeras and, therefore, by definition to subject matter that is not human. The chimeras of the present invention are not naturally occurring and were unknown prior to the present invention.

Second, the Supreme Court has held that embryos, even those consisting exclusively of human cells, are not constitutionally protected as human beings (*see*, *Roe v. Wade*, 410 U.S. 113 (1973)). Congress--in spite of almost thirty years of vigorous public debate--has indicated no intention of altering this holding. That holding is mandatory authority and precludes the Examiner's

finding that a single cell is sufficient to make a human being. Embryos which are <u>not</u> exclusively human in origin, viz. the embryos of this invention, which contain human as well as animal cells, do not fall under 1077 OG 24 (4/21/87). Utility of such chimeric embryos as experimental models in biomedical and developmental biological research was documented in the original application. These embodiments of the invention are appropriate subject matter for protection under 35 U.S.C. § 101.

Third, as noted by the Examiner, mice and sheep have been engrafted with human bone marrow cells, and have been raised in laboratories as subjects of scientific investigations (Pixley, et al., (1994) *Pathobiology* **62**, 238-44; Almeida-Porada, et al., (1996) *Exp. Hematol.* **24(3)**, 482-7).

Pixley, et al., established long term chimerism in normal mice transplanted *in utero* with human fetal hematopoietic stem cells. These human cells were injected into fetal mouse peritoneal cavities on days eleven through thirteen of gestation. These animals may develop and contain human cells in various organs. This engraftment of human cells into mouse fetuses does not now qualify the mouse as a human being, nor does it create a human being. The Office has never held that it does prior to the present invention and has regularly granted patents on such inventions.

Almeida-Porada, et al., describes the transplantation *in utero* of preimmune fetal sheep with human hematapoietic stem cells which result in a long term chimerism. These experiments reported the long term persistence of human cells in the human/sheep zenograft model. As with the above, the sheep, although containing human cells, are not considered human beings.

While Applicant disputes the Examiner's claim that these studies represent prior art with respect to the present invention (see below), it is clear that these organisms represent "animals containing human cells." They are not constitutionally protected as human beings. They have no

civil rights. They have no constitutionally recognized right of "reproductive choice." Because specific utility of non-human animals containing human cells, constructed by the methods of this invention, was documented in the original application, such organisms do not fall under 1077 OG 24 (4/21/87).

Applicant respectfully submits that the PTO is confusing the issue of chimeric embryo, chimeric cell line, and chimeric animal with that of grafts or transplants of tissue or organs between species. The claimed chimeric cell lines and chimeric animals originate from chimeric embryos. If a chimeric embryo develops with 99% human cells and 1% non-human primate cells, the resultant embryo is not human. It is not analagous to transplanting a non-human organ or tissue into a human being resulting in an isolated concentration of non-human cells. In a developing embryo even 1% non-human primate cells will contribute to the development of the entire chimeric embryo, thus making it non-human.

Applicant respectfully submits that a proportion of human cells in an organism does not make that organism a human being. Applicant does not claim a human being but, rather, a chimeric embryo, chimeric cell line, or chimeric animal, derived or isolated from the chimeric embryo. The fact that a chimera has a human cellular component cannot exclude it from patentability, any more than it did in the numerous patents that share that feature and have been awarded by the PTO. Subject matter consisting of, or derived from, human cells in non-human animal systems has been, and continues to be, patentable.

Attached as Exhibit "A" are copies of several patents that have issued, that would apparently "embrace a human being". While each application is evaluated on its own facts, the PTO must

provide a consistent interpretation as to what is patentable subject matter and what is not. Inventors routinely consult issued patents for guidance in determining the patentability of their own inventions.

II. The Claims Define Patentable Subject Matter

Each of the claims has been rejected under Sections 102 and/or 103 over a combination of one or more of nine references. If, as the Examiner contends, the claimed invention was anticipated and/or obviated by one or more of these references, the multiplicity and complexity of combinations of them would be unnecessary. In fact, none shows each element of the claimed subject matter, either alone or in combination. Perhaps more important, the unpredictability of the science--one of the benefits of the present invention--belies the conclusion that the invention as a whole would have been known by or obvious to one of ordinary skill in the art.

A. The Claims are Patentable over Zanjani et al. or Almeida-Porada et al. or Pixley et al.

Claims 1, 2, 5-7, 13, 16, 38-48, 53, and 54 are rejected under U.S.C. § 102(b) as being anticipated by Zanjani et al. (1996) *Int. J. Hematol.* **63**, 179-192 or Almeida-Porada et al. (1996) *Experimental Hematol.* **24**, 482-487. Claims 1, 2, 5-7, 13, 16, 28, 29, 32-34, 38-48, 53, and 54 are rejected under 35 U.S.C. § 102(b) as being anticipated by Pixley et al. (1994) *Pathobiol.* **62**, 238-244. These rejections are respectfully traversed.

The Examiner states that the invention was anticipated by the description by Zanjani et al. (1996) and Almeida-Porada et al. (1996) of the introduction of human hematopoietic cells into sheep *in utero*, and by Pixley et al. (1994) of the introduction of human hematopoietic cells into mice *in utero*. Zanjani and his associates (Flake, A. W., and Zanjani, E. D. (1993) *In utero* Transplantation of Hematopoietic Stem Cells. *Crit. Rev. Oncol. Hematol.* **15,** 35-48; Pixley et al. (1994); Zanjani et

al. (1996); Almeida-Porada et al. (1996); Pixley, J. S., Zanjani, E. D., Shaft, D. M., Porada, C., and Mackintosh, F. R. (1998). Prolonged Hematopoietic Chimerism in Normal Mice Transplanted *in utero* with Human Hematopoietic Stem Cells. *Pathobiology* **66,** 230-9) conducted late embryo grafting experiments to produce hematopoietic organisms, i.e., mixtures of blood forming cells in an organism (a sheep or a mouse) that is unambiguously of one species.

The Examiner contends that these organisms are chimeras, *i.e.*, "an organism made up of two or more tissues of different genetic composition". The Examiner contends that these organisms have composite morphology because their structure is clearly from two distinct sources. Applicant maintains that the organisms in these cited references do not fall within any generally accepted definition in the art of a chimera. The organisms of Zanjani, et al., Almeida-Porada, et al. and Pixley, et al. are not true chimeras.

Fehilly, et al., (1984) and Meinecke-Tillmann and Meinecke (1984) described true embryo chimeras that exhibit composite morphology and multi-tissue chimerism. This definition is generally accepted in the field by one of ordinary skill in the art. True embryo chimeras do not result from xenografts or transplants of adult or differentiated cells. The organisms produced by Zanjani, et al., Almeida-Porada, et al., and Pixley, et al., do not exhibit composite morphology and multi-tissue chimerism of true embryo chimeras. The work of the Zanjani et al., Almeida-Porada et al., or Pixley et al. groups represents xenograft models. None anticipates the claimed invention.

The PTO is defining chimera as "an organism made up of two or more tissues of different genetic composition" (Office Action at p. 9). Applicant respectfully rejects this definition. The

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PTO's definition would have any graft or transplant patient considered a chimera. This is clearly not the case.

Dorland's Medical Dictionary defines a "chimera" as "an individual organism whose body contains cell populations derived from different zygotes of the same or different species, occurring spontaneously, as in twins (blood group chimeras) or produced artificially, as an organism which develops from combined portions of different embryos, or one in which tissues or cells from another organism have been introduced." Although some investigators, such as Almeida-Porada, et al., (1996), and Brustle, et al., (1998), both cited above, refer the organisms they generated as "chimeras" or "chimeric," they clearly meant this in the sense of "one in which tissues or cells from another organism have been introduced." In the case of Almeida-Porada, et al., (1996) sheep fetuses (not embryos) were transplanted with fetal or adult human cells, and in the case of Brustle, et al., (1998), embryonic rats received human fetal (not embryonic) brain cells. In each of these cases, and in all other analogous cases in the literature, such as Pixley, J. S., Zanjani, E. D., Shaft, D. M., Porada, C., and Mackintosh, F. R. (1998). Prolonged Hematopoietic Chimerism in Normal Mice Transplanted in Utero with Human Hematopoietic Stem Cells. *Pathobiology* **66,** 230-9, there is no question of the resulting organism being anything but a sheep, rat, or mouse containing some human cells in a particular organ—bone marrow or brain.

The present invention claims a "chimera" in Dorland's first sense of "an organism which develops from combined portions of different embryos." None of the citations in the literature involving human-animal "chimeras" at the time of filing involved embryo aggregation chimeras of the type produced between sheep and goats by Meinecke-Tillmann, S., and Meinecke, B. (Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307,**

637-8; 1984) and Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (Interspecific chimerism between sheep and goat. *Nature* **307,** 634-6; 1984). In the widely-used textbook *Developmental Biology* (Sinauer, 1997), well known cell biologist Scott Gilbert defines "chimeric mice" as "the result of two or more early cleavage (usually 4- or 8-cell) embryos that have been artificially aggregated to form a composite embryo." These embryo aggregation chimeras, which as Gilbert points out, can also be produced with mixtures of embryo cells and embryo stem (ES) cells, clearly correspond to our invention, and not to the animals generated by Almeida-Porada, et al., (1996) and Brustle, et al., (1998).

The present invention describes chimeric embryos containing human cells, where aggregation of **totipotent** cells (i.e., blastomeres or ES cells) of two or more species is performed. This is entirely different, and leads to different developmental outcomes, than the engraftment of **multipotent** stem cells during fetal stages.

Applicant respectfully submits that Zanjani et al., Almeida-Porada et al., or Pixley et al. fail to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections is respectfully requested.

B. The Claims are Patentable over Cheng et al., or Catalog of Cell Lines and Hybridomas.

Claims 10-12 and 50-51 are rejected under 35 U.S.C. § 102(b) as being anticipated by Cheng et al. (1994) *Develop.* **120**, 3145-3153. Claims 10-12, 49, and 51 are also rejected under 35 U.S.C. § 102(b) as being anticipated by Catalog of Cell Lines and Hybridomas, 7th ed., American Type Culture Collection (ATCC), Rockville, MD. 20852-1776, 1992, entry HTB 157, HTB 158, and HTB 160, page 271. The Examiner states that the description of mouse primordial germ cells by

Cheng et al. anticipates Claims 10-12 and 50-51 of the invention, as does the existence of human cell lines anticipates Claims 10-12, 49, and 51 in the American Type Culture Collection (Office Action at pp. 10-11). These rejections are respectfully traversed.

Cells derived from chimeras are known to differ in immunological properties from equivalent cells in non-chimeric animals. One of ordinary skill in the art would expect that this would also likely pertain to cell lines derived from chimeras. Applicant does not completely understand the PTO's continued rejection under 35 U.S.C. § 102(b) as being anticipated by Chang, et al. and the American Type Culture Collection Catalogue of Cell Lines. In an immunological assay one would only be concerned with the cells bearing immunologic capacity. As for cells not involved in immune responses, such as hepatocytes or epithelial cells, the stimulation of an immune response in other cells by these hepatocytes or epithelial cells would also be valuable in studying the development of the chimeric embryos, chimeric cell lines, and chimeric animals. Much could be learned from comparison of the properties of cell lines derived from the human/non-human chimeras of the present invention with cell lines derived from non-chimeric embryos or organisms, such as those described by Cheng et al.

Embryo aggregation chimeras produced according to an embodiment of the present invention would be expected to be immunologically unprecedented in that they would likely be tolerant to grafts from both human and the nonhuman species used, but not from other species. For example, Gustafson, et al., (1993) tested four sheep-goat chimeras with a goat or sheep sibling having an identical genotype to one of the two component species of cells for tolerance through mixed lymphocyte response (MLR) and skin grafts. None of the four chimeras showed a response to its sibling in MLR and three of the four accepted sibling skin grafts. This demonstrates that the

chimerism exhibited by these animals was sufficient to render the chimera tolerant to antigens expressed by the sibling (Gustafson, R. A., Anderson, G. B., BonDurant, R. H., and Mahi-Brown, C. (1993). Tolerance of Sheep-Goat Chimeras to their Component Cells. *J Reprod Immunol* **23,** 155-68). In a commonly used model of interspecies grafting, the athymic, or nude, mouse, grafts from other species are tolerated, but only because the mouse's cellular immunity is entirely compromised. The chimeric model of the present invention is different—there is tolerance to the originating species because the relevant antigens were present during development. This model would therefore be highly useful in understanding the genesis of the tissue immune response in humans, and accelerate the development of anti-graft rejection human therapeutics. This indicates that lymphocytes, and probably Langerhans dendritic cells (and any cell lines derived from them) prepared from chimeras would have different patterns of gene expression from corresponding populations of either of the originating (human and nonhuman) species.

The Examiner also states that "the animals that are prepared from the chimeras may not harbor any transgenes if the germ cell from which the animals derive did not harbor a transgene" (Office Action at p. 11). Applicant respectfully maintains that this is incorrect. With the exception of Claims 16-18, which refer to descendants of chimeric animals, all animals referred to in the claims are "developed" from chimeric embryos, which means that they are not the result of reproductive breeding. The presence or absence of transgenes in germ cells is irrelevant.

Applicant maintains that if the chimeric embryo were "mosaic" as the Examiner contends, then the chimeric cell line generated from that chimeric embryo would also be mosaic, which by definition means that some cells would include the transgene. Although not every chimeric cell line derived from a chimeric embryo produced according to this invention would have a transgene

introduced during the chimeric embryo's construction, populations of the chimeric embryo's cells which contained the transgene could be readily identified by the polymerase chain reaction (PCR). Then if desired, cell lines from the same chimeric organism with or without the transgene could be generated. Applicant, in the desire to move the application towards issuance, has canceled all claims concerning the presence of transgenes in the chimeric embryo, chimeric cell line, and chimeric animal.

Applicant respectfully submits that Cheng et al. or American Type Culture Collection fail to disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections is respectfully requested.

C. The Claims are Patentable over Bradley et al..

Claims 13-18, 53, and 54 are rejected under 35 U.S.C. § 102(b) as being anticipated by Bradley et al. (1992) *Bio/Technology* **10**, 534-539. This rejection is respectfully traversed.

The Examiner states that the description of transgenic mice by Bradley et al. anticipates Claims 13-18, 53, and 54 of the invention. The animals of Claims 13-15, however, are **developed from chimeric embryos**. Fehilly et al. (1984) and Meinecke-Tillmann and Meinecke (1984) disclose that the form, appearance, and biology of any such animals would be very unlike the non-chimeric transgenic animals described by Bradley et al., which in contrast would be immediately identifiable as to species. The Examiner has cited no evidence to the contrary. With regard to the progeny or descendants of chimeric animals, claimed in Claims 16-18, although these may themselves be non-chimeric, their immunological and reproductive properties inevitably would be altered by the process of being bred from chimeras, as described above, and therefore would be biologically distinct from progeny or descendants of non-chimeric animals.

The Examiner contends that the claims are not clearly directed to a chimeric animal, but merely an animal derived from a chimeric embryo, and maintains that the animal derived is not necessarily chimeric. If the claim is read to encompass the descendant of the chimeric embryo, in the Examiner's view, the animal is not chimeric but is of the species that contributed to the germ cell. The Examiner contends that a chimeric embryo encompassed by the claims may be indistinguishable from an animal of the prior art because the recited human cells are not required to contribute to any particular tissue or tissues and may actually contribute very little to the animal as a whole and, as such, the animal would be indistinguishable from the animal of the second species.

It is well-known from studies with embryo aggregation chimeras that there is variability in the degree of chimerism obtained with the technique (Jaszczak, K., Parada, R., and Guszkiewicz, A. (1999). Cytogenetic Study of Some Tissues and Age-Related Changes in Cell Proportions in a Goat-Sheep Chimera. *Cytogenet Cell Genet* **84,** 55-7; MacLaren, L. A., Anderson, G. B., BonDurant, R. H., and Edmondson, A. J. (1992). Inter- and Intraspecific Placentae in Sheep, Goats and Sheep-Goat Chimeras. *J Comp Pathol* **106,** 279-97). Rather than being an impediment to using this kind of organism in research, it has provided an advantage. For example, MacLaren et al. (1992) established goat and sheep pregnancies in sheep, goats, and chimeras, and found that goat pregnancies in chimaeras generally terminated before timed samples could be obtained, both normal and abnormal placentation occurred in chimeras pregnant with lambs. They concluded that "the physiological events that regulate implantation are different in the two species, despite anatomical similarities between the ovine and caprine placenta." By correlating the degree of chimerism of the mother with the extent of aberrant placentation, general principles concerning placentation can be obtained from such studies. For the human, where there is no possibility of directly studying

placentation, the availability of a chimeric human-nonhuman primate model would be exceptionally useful.

Applicant acknowledges that Claims 13-18 and 52-55 were not directed to a chimeric animal but merely an animal derived from a chimeric embryo. Applicant has amended these claims to be directed to a chimeric animal. Applicant believes the claims in their previous format should be allowable, but makes these amendments in the interest of placing the application in better form for issuance.

Applicant respectfully submits that Bradley et al. does not disclose the subject matter of the claimed invention. Reconsideration and withdrawal of these rejections are respectfully requested.

D. The Claims are Patentable over Gustafson et al.

Claims 1, 2, 5-7, 19, 20, 23-25, 28, 29, 32-34, and 37-48 are rejected under 35 U.S.C. § 103 as being unpatentable over Gustafson, et al., (1993) *J. Reprod. Fert.* **99**, 267-273. This rejection is respectfully traversed.

The Examiner cites Gustafson, et al., as disclosing the ability of sheep-goat chimeras to gestate sheep-goat hybrid conceptuses and states that producing human/non-human chimeras to perform similar studies would therefore have been obvious. Interspecific chimeric mammalian embryos have been described at least since 1973 (Stern, M. S. (1973) Chimaeras Obtained by Aggregation of Mouse Eggs with Rat Eggs. *Nature* **243**, 472-3), interspecific-*intra*generic chimeric mammalian organisms at least since 1982 (Rossant, J., Croy, B. A., Chapman, V. M., Siracusa, L., and Clark, D. A. (1982) Interspecific Chimeras in Mammals: A New Experimental System. *J. Anim. Sci.* **55**, 1241-8), and interspecific-intergeneric chimeric mammalian organisms at least since 1984 (Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984) Interspecific Chimerism Between Sheep

and Goat. *Nature* **307**, 634-6; Meinecke-Tillmann, S., and Meinecke; B. (1984) Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307**, 637-8). Yet, none of this substantial body of published research, or subsequent teachings, including Gustafson, et al., (1993) taught or suggested the claimed invention: chimeric embryos, cell lines, and animals **containing human cells**; or the specific beneficial uses recited in the specification for directly studying **human** developmental biology, physiology, and toxicology.

Applicant submits that it would not be obvious under Gustafson, et al., for one of ordinary skill in the art at the time of the invention to make a cell aggregate comprising human cells. The Examiner contends that an invention is capable of being unpredictable, yet obvious. The Examiner also contends that the unpredictability is based upon the ability of the embryo to form a cooperative entity. The Examiner contends that from the teaching of Gustafson, et al. that it would be obvious of one of ordinary skill in the art at the time of the invention to make a cell aggregate comprising human cells, even if the resultant viability was unpredictable. Applicant maintains that Gustafson, et al. does not provide the motivation or teaching to make chimeric embryos with human cells.

The Examiner asserts that Gustafson, R. A., Anderson, G. B., BonDurant, R. H., and Sasser, G. R. (1993). Failure of Sheep-Goat Hybrid Conceptuses to Develop to Term in Sheep-Goat Chimaeras. *J Reprod Fertil* **99,** 267-73 provided the motivation to make chimeric embryos containing human cells. The abstract of that paper is as follows:

> Six hybrid pregnancies were established: three in sheep-goat chimaeras, one in a sheep-(sheep-goat)hybrid chimaera and two in does. Pregnancies were monitored weekly by ultrasonography and peripheral concentrations of pregnancy specific protein B (PSPB) were measured. Placental development as detected by ultrasonography appeared to be slower in hybrid-in-goat pregnancies than in hybrid-in-chimaera pregnancies, although this difference was

> not reflected in PSPB concentrations. Time of fetal death could not be predicted from PSPB concentrations. Chimaeras appeared to carry hybrid pregnancies longer than ewes and does usually carry hybrid pregnancies, but none was carried to term.

Although this paper utilized chimeras to study pregnancy retention and placental development, i.e., some of the things that might be studied if chimeric animals containing human and nonhuman cells were available, as per an embodiment of the present invention, the issues and discussion are very far from anything involving human biology. This paper has been cited twice in the literature indexed in the Scientific Citations Index ("SCI") since the time it was published. These citations are: Slavik, T., Kopecny, V. and Fulka, J. (1997) Developmental Failure of Hybrid Embryos Originated after Fertilization of Bovine Oocytes with Ram Spermatozoa. *Molec. Reprod. Develop.* **48**, 344-349, and Willard, S. T., Sasser, R. G., Jaques, J. T., White, D. R., Neuendorff, D. A., and Randel, R. D. (1998). Early-pregnancy Detection and the Hormonal Characterization of Embryonic-Fetal Mortality in Fallow Deer (Dama-Dama). *Theriogenology* **49**, 861-869. Neither reflects the motivation attributed to Gustafson, et al., by the Examiner.

Applicant submits that Examiner's statement "However, as stated in the previous Office Action, Gustafson, et al., provided the motivation to make chimeric embryos with human cells" fails to identify any such motivation. Persons who have relied upon Gustafson have not been so motivated. The Examiner has provided no reference that teaches or suggests chimeric embryos containing human cells. Examiner maintains the rejection based upon the ability of the embryo to form a cooperative entity. Applicant submits that there was actually a teaching away of the formation of chimeric embryos containing human cells. The only references relying upon Gustafson did so in the context of embryo mortality, not formation. Gustafson, et al., does not disclose the

subject matter of Claims 1, 2, 5-7, 19, 20, 23-25, 28, 29, 32-34, and 37-48. No reference exists that would have made the present invention obvious. Reconsideration and withdrawal of these rejections is respectfully requested.

E. The Claims are Patentable over Watanabe et al., in view of Robertson, et al.

Claims 1, 8, 9, 19, 26-28, 35-36, and 37-48 are rejected under 35 U.S.C. § 103 as being unpatentable over Watanabe et al. (1992) *Develop.* **114**, 331-338 in view of Robertson, et al., (1986) *Nature* **323**, 445-448. This rejection is respectfully traversed.

Neither Watanabe, et al., either alone or in combination, or Robertson, et al., disclose the present invention. Neither discloses chimeric embryos and animals **containing human cells**, let alone the specific uses in directly studying **human** developmental biology, physiology, and toxicology described in the present specification.

Watanabe, et al., and/or Robertson, et al., do not provide the motivation or a reasonable expectation of success in making chimeric embryos. The Examiner concedes that these references do not disclose chimeric embryos containing human cells. The knowledge necessary to make the present invention was not within the level of ordinary skill of the art at the time the claimed invention was made.

The Examiner asserts that Watanabe, et al., (1992) Distribution Analysis of Transferred Donor Cells in Avian Blastodermal Chimeras. *Development* **114,** 331-8, in view of Robertson, E., et al., (1986). Germ-line Transmission of Genes Introduced into Cultured Pluripotential Cells by Retroviral Vector. *Nature* **323,** 445-8 provide motivation or a reasonable expectation of success in making chimeric embryos containing human cells. The Watanabe, et al., (1992) paper addresses

chicken embryos, which are extremely different from mammalian embryos in morphology. Chicken embryos exhibit blastodermal development rather than arising from a morula and inner cell mass, and in gestational environment, developing in a bird egg rather than a mammalian uterus. This paper was cited 5 times in the literature indexed in the SCI. Not one of these papers extrapolated from the teaching of Watanabe, et al., to the expectation of success in producing mammalian embryo chimeras. Robertson, et al., deals with introducing transgenes into mouse ES cells. The present invention utilizes existing art, including the teachings of Robertson, et al., and does not claim the technique of introducing transgenes into mammalian ES cells as innovative. Beyond disclosing that technique, nothing in the reference provides a motivation for making chimeric embryos containing human cells. This paper was cited 58 times per the SCI between 1997 and 1999. None of the papers cited discussed or mentioned the generation of chimeric embryos containing human cells, even in the most speculative portions of the discussions.

Of the 58 papers citing Robertson, et al., during this period, two papers were reviews presenting an overview of the field of stem cell and embryo manipulation research and prospects for the future. These were: Moreadith, R. W., and Radford, N. B. (1997). Gene Targeting in Embryonic Stem Cells: The New Physiology and Metabolism. *J Mol Med* **75**, 208-16, and Brinster, R. L. (1998). Embryo Culture, Stem Cells and Experimental Modification of the Embryonic Genome. An interview with Professor Ralph Brinster. *Int J Dev Biol* **42**, 861-78. Interestingly, while the review of Moreadith and Radford states, "Optimally of course cardiovascular phenotypes would be interrogated in the intact animal," and it cites an early report of the isolation of a human ES-like cell line (Bongso, A., Fong, C. Y., Ng, S. C., and Ratman, S. (1994). Isolation and Culture of Inner Cell Mass Cells from Human Blastocyst. *Hum Reprod* **9**, 2110–2117), there is no suggestion in this

article that an obvious or even possible use of human ES cells would be to construct a human-nonhuman chimeric embryo, cell line, or animal for experimental purposes.

Thus, even in these two broad overviews, containing speculations on future directions of the field of gene targeting, stem cells, and embryo research, there is no mention of prospects of generating embryo aggregate chimeras containing human cells of the present invention. Rather, the art suggests avoidance, or a "teaching away" from the invention in the scientific literature. A composition of matter containing nontransgenic or transgenic human embryonic or ES cells, along with cells of a nonhuman animal, was never proposed, despite prior teaching that made it technically possible.

Applicant respectfully submits that these references provide no motivation, teaching, or suggestion for making chimeric embryos containing human cells. Had these references provided the motivation inferred by the Examiner to create chimeric embryos containing human cells, experiments to that end would have been reported over the last eight years, from the date of the Watanabe, et al., reference.

Applicant respectfully submits that Watanabe et al. and Robertson, et al., do not disclose the subject matter of Claims 1, 8, 9, 19, 26-28, 35-36, and 37-48. Reconsideration and withdrawal of these rejections are respectfully requested.

Although Applicant believes that the claims in present form contain patentable subject matter, Applicant has added additional Claims 60-69 claiming uses of the chimeric embryos containing human cells. Applicant submits these new claims in the interest of moving the application to issuance.

III. <u>The Claims Satisfy 35 U.S.C. § 112, First Paragraph</u>

Claims 39-48 and 55 are rejected under 35 U.S.C. § 112, First Paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor, at the time the application was filed, had possession of the claimed invention. The Examiner asserts that the specification does not provide explicit or implied support for the claimed embryos which are viable for no longer than a specific time or which are terminated at a specific time. In addition, the Examiner asserts that the specification does not provide support for newly advanced Claim 55 which requires that a resultant animal display a specific phenotype.

Claims are rejected that refer to "viability" of the chimeric embryo. Viability of an embryo or fetus in experimental embryology refers to its ability to continue developing *in vitro* (Gardner, D. K. 1999. Development of Serum-Free Culture Systems for the Ruminant Embryo and Subsequent Assessment of Embryo Viability. *J Reprod Fertil Suppl* **54,** 461-75) or *in utero* (e.g., Arkaravichien W. and Kendle K. E. (1992) Fetal Viability and Fetal Growth after Prolonged Uterine Contractions Induced by Progesterone Withdrawal in Late Pregnancy in Rats. *J Reprod Fertil* 96, 299-308). Claims 39-43 refer to those embryos and fetuses that continue developing for no more than the specified periods. It is well known from the experimental literature that viability of experimentally manipulated embryos depends on a variety of subtle factors (Matsumoto, K., Miyake, M., Utsumi, K., and Iritani A. (1989) Production of Identical Twins by Separating Two-Cell Rat Embryos. *Gamete Res* **22,** 257-63). Since there is no way to predict *a priori* how long any human-nonhuman animal chimeric embryo will develop *in vitro* or *in utero*, these claims are in recognition of the usefulness of those embryos which for spontaneous reasons do not develop to term. It is reasonable

to foresee that such chimeric embryos could be commercially supplied to medical researchers with a declaration that the extent of further development will be variable. Patent protection for such a product would not thereby be precluded. There is no requirement for these claims that the period of viability will be known in advance.

Claims that use the term "terminated" were also rejected. However, termination has a very specific meaning in experimental embryology, referring to experimenter-initiated cessation of development for evaluation purposes. Thus Price et al. write: "Dams and their fetuses were evaluated for evidence of maternal or developmental toxicity, as reported previously. At termination on gd 20, maternal whole blood was collected..." Price, C. J., Strong, P. L., Murray, F. J., and Goldberg, M. M. (1997) Blood Boron Concentrations in Pregnant Rats Fed Boric Acid Throughout Gestation. *Reprod Toxicol* **11**, 833-42, and Field et al. write: "Following termination (gd 17, mice; gd 20, rats) fetuses were examined for external, visceral, and skeletal malformations." (Field, E. A., Price, C. J., Sleet, R. B., Marr, M. C., Schwetz, B. A., and Morrissey, R. E. (1990). Developmental Toxicity Evaluation of Acrylamide in Rats and Mice. *Fundam Appl Toxicol* **14**, 502-12). Since chimeric embryos harvested at specified times after gestation has been initiated will be useful to investigators in scientific and medical fields, for histological and tissue culture studies, for example, Claims 44-48 are in recognition of the usefulness of those chimeric embryos and fetuses that do not develop to term because of the intervention of the supplier.

Applicant, on Page 5 of its original application, states "Once chimeric embryos are produced they can be propagated for varying periods of time in culture, where they may undergo a series of developmental steps . . . For some uses, the embryos can be brought to term, forming the chimeric

animals of the invention." Applicant respectfully submits that it is well known in the art that chimeric organisms may or may not cease to be viable at any given time.

Claims 1-36 and 37-55 are rejected under 35 USC § 112, First Paragraph for lack of adequate enabling disclosure. The Examiner asserts that the specification fails to provide an enabling disclosure for how to make and use the claimed invention. This rejection is respectfully traversed. Applicant respectfully submits that the claim amendments and remarks below obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

The studies discussed in Applicant's response to the initial Office Action (Amendment and Response dated June 16, 1999, at pp. 17-23) establish that it was known in the published literature that the technology for producing chimeric mammalian embryos is "robust," i.e., insensitive to variations in procedure, species origin of the cells, or species origin of the zona pellucida. Techniques developed for mouse embryo culture, sheep embryo culture, mouse-mouse, and mouse-rat chimeras, have all proved successful for sheep-goat chimera production, and even the biological materials of the culture environment may be derived from a third species, with no detriment to the outcome. Applicant respectfully submits that there is absolutely no indication from the scientific literature that use of a different mammalian species, such as the human, would require "undue experimentation" for the design of a protocol for producing chimeras. Rather, the references cited by the Examiner reflect the utility of these techniques across species. Applicant therefore respectfully submits that using the cited references, the present invention is adequately enabled.

The Examiner asserts that the demonstration of viable "geeps" does not enable the preparation of other viable interspecific chimeras. However, it is commonplace in mammalian developmental biology that once an experimental protocol is established in one species the

technology is transferable to other species. For example, although it was not generally considered that mammalian embryos could be cloned using somatic cell nuclei, once this was performed in sheep in 1997 (Wilmut, I., Schnieke, A. E., McWhir, J., Kind, A. J., and Campbell, K. H. (1997). Viable Offspring Derived from Fetal and Adult Mammalian Cells. *Nature* **385,** 810-3), the same technique was used to clone mice (Wakayama, T., Perry, A. C., Zuccotti, M., Johnson, K. R., and Yanagimachi, R. (1998). Full-term Development of Mice from Enucleated Oocytes Injected with Cumulus Cell Nuclei. *Nature* **394,** 369-74) and cows (Cibelli, J. B., Stice, S. L., Golueke, P. J., Kane, J. J., Jerry, J., Blackwell, C., Ponce de Leon, F. A., and Robl, J. M. (1998). Cloned Transgenic Calves Produced from Nonquiescent Fetal Fibroblasts. *Science* **280,** 1256-8) the following year, and more recently, pigs. The major reason for this is that mammalian embryos are quite similar to one another in morphology and cellular biochemistry through the morula stage.

In a recent retrospective of the scientific field of early mammalian embryo manipulation and chimera production, Richard Gardner, one of the pioneers in this field, discusses his experience in adapting techniques originally developed in one mammalian species to work in other species (Gardner, R. L. 1998. Contributions of Blastocyst Micromanipulation to the Study of Mammalian Development. *Bioessays* **20,** 168-80). He describes initial work with the rabbit embryo, then, encountering technical problems, continued the work with the mouse embryo (Gardner, R. L. 1968. Mouse Chimeras Obtained by the Injection of Cells into the Blastocyst. *Nature* **220,** 596-7), and then returning to the rabbit embryo, where he eventually obtained the chimeras he was seeking (Gardner, R. L., and Munro, A. J. 1974. Successful Construction of Chimaeric Rabbit. *Nature* **250,** 146-7). Gardner's techniques of blastocyst injection, or the alternative of blastomere aggregation, have been used to generate chimeric mice, rats, rabbits, hamsters, sheep, goats, and pigs. Most of these have

been intra-genus chimeras, but some have been inter-genus. While it is the case that embryos of individual mammalian species may differ in the timing of some developmental changes at the cellular and molecular levels (Tesarik, J. 1988. Developmental Control of Human Preimplantation Embryos: A Comparative Approach. *J In Vitro Fert Embryo Transf* **5,** 347-62), with regard to their behavior *in vitro,* where chimeric embryos are initially constructed, human blastomeres behave similarly and have similar nutritional requirements to the blastomeres of other mammalian species (FitzGerald, L., and DiMattina, M. 1992. An Improved Medium for Long-Term Culture of Human Embryos Overcomes the In Vitro Developmental Block and Increases Blastocyst Formation. *Fertil Steril* **57,** 641-7).

As the description of the invention includes chimeric embryos that are grown *in vitro* without implantation, and it is certain that an inter-genus chimera including human blastomeres will develop to the requisite extent to be useful according to the description. Moreover, there is no indication in the literature that an inter-genus chimera constructed from any two mammalian species will not develop to some extent *in utero*. Indeed, there is documentation that such chimeras (e.g., between mouse and rat) are highly informative objects of study even if they never develop to term (Gardner, R. L., and Johnson, M. H. (1975). Investigation of Cellular Interaction and Deployment in the Early Mammalian Embryo Using Interspecific Chimaeras Between the Rat and Mouse. *Ciba Found Symp* **29,** 183-200). Although the work on the experimental production of mammalian chimeras since the late 1960's, leading up to the two reports of "geeps" in 1984, provided the technical background to potentially construct a human-nonhuman chimera, not enough was known about early human embryos to enable the present invention. A survey of the Medline indexed literature since 1978, when the birth of Louise Brown, the first "test tube baby," was announced, indicates the acceleration

of research activity in this area that followed. Of the 4481 listed articles containing the term "human blastocyst," in the title, abstract, or keywords, 76 were published in 1978, 113 were published in 1984, the year the sheep-goat chimeras were announced, and 444 were published in 1997, the year this application was filed. Three quarters of these articles (3208) were published between 1984 and 1997. Thus, what was essentially speculative in 1984 was enabled by 1997 as a result of the vast increase in knowledge of the early human embryo and the means to manipulate it.

The Examiner cites "unpredictable outcomes" as a basis for rejecting the claims. All biotechnological procedures inherently lead to unpredictable outcomes. Genetic and other uncontrolled biological variability of organisms necessarily make outcomes unpredictable. This has not disqualified previously patented biological inventions, and should not disqualify the present invention. Moreover, it is not necessary or appropriate to require predictability as a condition of patentability. The degree of predictability of the present invention is consistent with the claimed utility of the subject matter of the invention.

It is not clear why the Examiner finds that the "unpredictable outcomes" of the present invention disqualifying. The level of predictability, reproducibility, or "quality control" requested by the Examiner is not appropriate and is not required for other similar inventions. The degree of predictability is a function of the claimed subject matter. It is particularly inappropriate to require a high degree of predictability in the present instance since, as described in the following paragraph, the variability of outcome is one of the useful aspects of our invention as a research tool.

Applicant maintains that the present invention cannot simultaneously be both unpredictable and obvious. As the references cited by the Examiner establish, the area of developmental biology

is unpredictable, making the utility of the present invention so powerful. It is for this reason, among others, that the claimed subject matter is not obvious.

Of the many papers published on intra-specific, inter-specific, and inter-generic mammalian chimeras, (e.g., Gardner, R. L. 1968. *Nature* **220**, 596-7; Rossant, J., Croy, B. A., Chapman, V. M., Siracusa, L., and Clark, D. A. 1982. *J Anim Sci* **55**, 1241-8; Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. 1984. *Nature* **307**, 634-6; Meinecke-Tillmann, S., and Meinecke, B. 1984. *Nature* **307**, 637-8), none found there to be a predictable ratio of cells from one or the other species in the chimeric embryos or animals that were generated from them. This lack of predictability does not impair the usefulness of the invention, since the degree of chimerism can be determined *ex post facto* and will be a relevant variable in many of the studies performed with these chimeric embryos and animals. Any embryos or organisms that result from our procedures which do not have evidence of both human or nonhuman cellular contribution to its tissues, which can be determined by karyotyping of lymphocytes from the fetal or newborn circulation (Jaszczak, K., Parada, R., and Guszkiewicz, A. 1999. Cytogenetic Study of Some Tissues and Age-Related Changes in Cell Proportions in a Goat-Sheep Chimera. *Cytogenet Cell Genet* **84,** 55-7), are not included in the claimed invention.

The Examiner refers to studies that document variations in the degree of chimerism obtained in sheep-goat studies, which depend on the ratio of cells of the different parental types in the original embryo, the degree of similarity of the genotype of the embryo to the gestational environment, and other uncontrolled factors. Several uses disclosed in the original application for embodiments of the present invention involve developing suitable models for developmental biological and immunological research (Application at pp. 18-21). The present invention will facilitate answering

scientifically and medically important questions concerning the survival of cells of one species in the environment of cells of another species, and the immunological tolerance of the gestational environment of nonhuman species for developing tissues and organs that contain human cells.

There is no *a priori* way to determine what proportion of the embryo will ultimately be due to each species' cells. After development has progressed, however, this would be easy to ascertain using species-specific molecular markers or "reporter" genes, as described in the application (Application at p. 8).

In developmental biological research there is no requirement for implantation of the chimeric embryo to obtain useful information on early development and interspecies compatibility. Much work is done *in vitro*. The survival rates of implanted chimeric embryos will be irrelevant for certain embodiments of the invention, and the invention provides a unique research tool in other embodiments. <u>The claimed invention is not limited to a specific degree or range of chimerism or chimeric lifetime</u>. All such variations are within the subject matter of the invention.

In this regard the inappropriateness of the additional requirement of predictability of outcome is stark. The degree of predictability necessary to eliminate undue experimentation is a function of the claimed subject matter and its utility. Experimentation need not be eliminated, rather, it must not be *undue*. With respect to the claimed subject matter, some degree of unpredictability is affirmatively desirable.

The Examiner also relies upon the possible "lack of fecundity of chimeric animals" as a basis for rejecting the claims. Fecundity was never an essential characteristic of the present invention. Even if fertile, chimeric animals would never breed true because by definition their germ lines would be mosaics of the two originating species. At best, a mating between chimeric animals could give

rise to offspring that were of one, or the other, originating species. Although some patented organisms are self-propagating, most compositions of matter are not. The inability to self-propagate is not a requirement for patentability under 35 U.S.C. § 101.

All the techniques specified by Applicant are from mammalian embryology, and the feasibility of producing human/non-human animal chimeras is justified by the success of these techniques using mammalian species more distantly related than humans and chimpanzees: i.e., rats and mice, and sheep and goats. As amended, Applicant's claimed invention relates to chimeras of human cells and cells of non-human **primates**.

The Examiner questions the use of ES cells in forming the chimeric embryos of the invention, stating that "[a]t the time of filing, the art regarded as unpredictable the obtaining of ES cells that would contribute to the germ line of the resultant animal" (Office Action at p. 16-17). The contribution of the ES cells to the germ line of the resultant animal is not a feature of the invention. Baribault et al., (1989) *Mol. Biol. Med.* **6**, 481-492, on the difficulties of assuring that ES cells will lead to germ line chimerism, is not relevant to the present invention.

The Examiner states, moreover, that true ES cells must contribute to the germ line. Although ES cells have been defined in various ways, ability to contribute to the germline has not been demonstrated in all cases in which the isolation of such cells has been reported. For example, in Iannaccone, P. M., Taborn, G. U., Garton, R. L., Caplice, M. D., and Brenin, D. R. (1994). Pluripotent Embryonic Stem Cells from the Rat are Capable of Producing Chimeras. *Dev Biol* **163,** 288-92, germline transmission was not demonstrated. Moreover, in Thomson, J. A., Kalishman, J., Golos, T. G., Durning, M., Harris, C. P., Becker, R. A., and Hearn, J. P. (1995). Isolation of a Primate Embryonic Stem Cell Line. *Proc Natl Acad Sci U S A* **92,** 7844-8, the authors state:

> Here we report the derivation of a cloned cell line (R278.5) from a rhesus monkey blastocyst that remains undifferentiated in continuous passage for > 1 year, maintains a normal XY karyotype, and expresses the cell surface markers (alkaline phosphatase, stage-specific embryonic antigen 3, stage-specific embryonic antigen 4, TRA-1-60, and TRA-1-81) that are characteristic of human embryonal carcinoma cells. R278.5 cells remain undifferentiated when grown on mouse embryonic fibroblast feeder layers but differentiate or die in the absence of fibroblasts, despite the presence of recombinant human leukemia inhibitory factor. R278.5 cells allowed to differentiate in vitro secrete bioactive chorionic gonadotropin into the medium, express chorionic gonadotropin alpha- and beta-subunit mRNAs, and express alpha-fetoprotein mRNA, indicating trophoblast and endoderm differentiation. When injected into severe combined immunodeficient mice, R278.5 cells consistently differentiate into derivatives of all three embryonic germ layers. When injected into severe combined immunodeficient mice, R278.5 cells consistently differentiate into derivatives of all three embryonic germ layers. **These results define R278.5 cells as an embryonic stem cell line,** to our knowledge, the first to be derived from any primate species. [Emphasis added.]

The criterion of contribution to the germline is not part of this definition.

Similarly, in the paper Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. (1998). Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* **282,** 1145-7, there is no evidence provided for germline transmission resulting from studies with these cells.

While it is true that germline transmission has been indicated by some investigators as a criterion for ES cells, that feature has nothing whatever to do with the ability of ES cells to contribute to a chimeric embryo. For example, Delhaise, et al., distinguish between chimeric mice produced with ES cells with germline transmission and those without germline transmission (Delhaise, F., Zhao, X., Bralion, V., Dessy, F., and Georges, M. (1993). Quantitative Estimation of Chimerism in Mice Using Microsatellite Markers. *Mol Reprod Dev* **34,** 127-32), and Mann and

Stewart discuss chimerism in mice produced using ES cells without considering germline transmission at all (Mann, J. R., and Stewart, C. L. (1991). Development to Term of Mouse Androgenetic Aggregation Chimeras. *Development* **113,** 1325-33).

Applicant respectfully submits that contribution to the germ line is not the defining characteristic of such cells, according to the scientific literature. For example, Wheeler (1994) describes the production and validation of swine ES cells with no recourse to tests of germ line chimerism (Wheeler MB (1994) Development and Validation of Swine Embryonic Stem Cells: A Review. *Reprod. Fertil. Dev.* **6**, 563-8). Wheeler reported the establishment of procine embryonic stem cell lines from preimplantation blastocysts and their ability to develop into normal chimeras. These ES cells could spontaneously differentiate into cystic embryoid bodies with ectodermal, endodermal, and mesodermal cell types. Culture of these ES cells to confluence or chemical induction of differentiation resulted in morphological differentiation into fibroblasts, adipocytes, and epithelial, neuronal, and muscle cells. The differentiation of these embryonic cell lines into several cell types indicates a pluripotent ES cell. Furthermore, chimeric swine have been successfully produced using these ES cells. The hallmarks used by Wheeler were: spontaneous differentiation into embryoid bodies; their differentiation into several cell types; and their capacity to contribute to chimeras when combined with normal embryo cells. Applicant submits that these hallmarks and definitions of ES cells would be understood by persons of ordinary skill in the art and adequately supports the claims of the present invention pertaining to the use of ES cells.

Non-human ES cells have been documented for the mouse (Martin, G. R. (1981) Isolation of a Pluripotent Cell Line from Early Mouse Embryos Cultured in Medium Conditioned by Teratocarcinoma Stem Cells. *Proc. Natl. Acad. Sci. USA* **78**, 7634-8), the pig (Wheeler (1994)), the

rhesus monkey (Thomson et al. (1995)), and the marmoset, another primate (Thomson JA, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP (1996) Pluripotent Cell Lines Derived from Common Marmoset (*Callithrix jacchus*) Blastocysts, *Biol. Reprod.* **55**,254-9), prior to filing the application. The statement by the Examiner fails to account for the teachings of these references. Armed with the background provided by this work, undue experimentation would not be required to practice the embodiments of the claimed invention pertaining to the production of chimeras from human embryo cells and non-human ES cells (Claims 6, 7, 10-29, 33-36). The mouse embryonic stem cells of these studies were established directly from normal preimplantation mouse embryos. The embryonic stem cells are pluripotent and were isolated from inner cell masses of late blastocysts. Eight embryonic stem cell lines from these studies were derived from common marmoset blastocysts. These embryonic stem cell lines were shown to differentiate into a number of different cell types. These teachings would have made the degree of experimentation required reasonable.

With regard to the generation of human ES cells, although not widely published at the time the application was filed, many groups were using the techniques employed for other mammals to develop human ES cell lines. During the year following filing of the present application, two groups published the isolation of such cell lines (Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. (1998) Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* **282,** 1145-7; and Shamblott, M. J., Axelman, J., Wang, S., Bugg, E. M., Littlefield, J. W., Donovan, P. J., Blumenthal, P. D., Huggins, G. R., and Gearhart, J. D. (1998) Derivation of Pluripotent Stem Cells from Cultured Human Primordial Germ Cells. *Proc. Natl. Acad. Sci. USA* **95,** 13726-31). These ES cell lines were isolated using existing techniques, without "undue experimentation." For example, in reporting the first primate ES cells

Thomson et al. stated: "The growth of monkey ES cells in culture conditions that support feeder-dependent human EC [embryonal carcinoma] cells **suggests that similar conditions may support human ES cells**," (Thomson et al. (1995) at p. 7848; emphasis added). That this understanding was correct was confirmed in the report by this group of the isolation of human ES cells, where it is stated: "five [human] ES cell lines originating from five separate embryos were derived, **essentially as described for nonhuman primate ES cells**," (Thomson et al. (1998) at p. 1145; emphasis added).

Prior to the time of filing at least one report appeared in the literature of an ES-like cell line derived from human embryos (Bongso, A., Fong, C. Y., Ng, S. C., and Ratnam, S. (1994). Isolation and culture of inner cell mass cells from human blastocysts. *Hum Reprod* **9,** 2110-7). This report was referred to by Moreadith and Radford (1997) in the following fashion:

> The advent of techniques to generate gain-of-function and loss-of-function mutations in laboratory animals represents one of the major accomplishments in cell and molecular biology in mammals over the past two decades. Although the technology is generally limited only to the mouse at present, substantial effort is underway to develop these techniques, and to refine existing techniques, in other species. Putative pluripotential ES cell lines have been derived in a number of other species including hamster [70], pig [71–75], sheep [73], cattle [76], rabbit [77], rat [78], mink [79], monkey [80], and even humans [81]. Thus it seems likely the technology will be advanced into these additional species over the next few years, and each one of these may lend itself uniquely to problems ranging from development to tissue and organ physiology.

Reference [81] is Bosgso et al. (1994). Moreover, it was generally known in the developmental biology community as of late 1997 that the Thomson group at the University of Wisconsin was working towards the isolation of human ES cells (their paper reporting this was published in 1998, and their 1995 paper reporting a primate stem cell line stated: "The growth of

monkey ES cells in culture conditions that support feeder-dependent human EC [embryonal carcinoma] cells **suggests that similar conditions may support human ES cells**" (Thomson, 1995, p. 7848; emphasis added). Taken together, these reports and the discussion of Bosgso et al. (1994) by Moreadith and Radford (1997) indicates that by late 1997 knowledge of human ES cells and anticipation of some of their uses (not including the generation of human-animal chimeras) was available to researchers. Applicant respectfully contends that the suggestion by the Examiner that existing art was insufficient at the time of filing to permit one of ordinary skill to characterize the properties and uses of anticipated human ES cells without undue experimentation is not correct.

The Examiner contends that chimeras prepared from intraspecific mice are not persuasive to show that the claimed invention is enabled, because the chimeras are made of mouse-mouse and the procedure cannot be extrapolated to include interspecific chimeras. Applicant maintains that procedures used to create intraspecific mice can be extrapolated to include chimeras made with animals of two different species. The Examiner contends that very few species have been demonstrated as useful in making interspecies chimeras and that one of skill in the art would not readily assume this to mean that any species of animal could likewise be successful. Applicant provides evidence below that the technology of creating chimeric embryos is robust, including interspecies chimeras between mouse, rat, sheep, and goat.

The Examiner suggests that the technology of generating interspecific chimeric embryos would be considered unlikely to succeed by one skilled in the art. On the contrary, Prather et al. reviewed this field in 1989 (Prather, R. S., Hagemann, L. J., and First, N. L. 1989. Preimplantation

Mammalian Aggregation and Injection Chimeras. *Gamete Res* **22**, 233-47), and stated in the overall summary of their findings:

> The preimplantation embryo is highly resilient to experimental manipulations. A specific manipulation that has revealed many clues to the developmental process is chimera production. Chimeras have been used to describe the importance of developmental characteristics of embryonic cells and how these characteristics are involved with developmental fate. These characteristics have been monopolized in the production of interspecific chimeras and the production of transgenic animals. This review attempts to discuss the major factors affecting preimplantation mammalian embryo chimera production.

A search of the entire Medline database using the terms: chimera and/or interspecies, interspecific, did not identify a single reference contradicting this expectation. In particular, there has not been a single report of an unsuccessful attempt to achieve some degree of informative development from an interspecifiic chimera. Thus, while rat-mouse chimeras do not develop into autonomous animals, the development that they undergo is highly informative. Thus, Gardner and Johnson (Gardner, R. L., and Johnson, M. H. 1975. Investigation of Cellular Interaction and Deployment in the Early Mammalian Embryo Using Interspecific Chimaeras Between the Rat and Mouse. *Ciba Found Symp* **29,** 183-200) write:

> [I]nterspecific chimaeras have been produced between rat and mouse embryos in which cells of the two species can be identified in sectioned embryos by immune fluorescence. These chimaeric embryos have been used to study differentiation of the trophoblast and inner cell mass, and the deployment of cells during morphogenesis. Preliminary results suggest that the two tissues are determined by the blastocyst stage, and that the trophoblast forms part of the extra-embryonic membranes originally presumed to be derived from the inner cell mass. Also, rat inner cell mass cells can induce mitosis in mouse trophoblast. Furthermore, the distribution of rat cells in implanted embryos suggests that the embryo may grow in a coherent clonal manner from a very early stage.

It should be noted that rat and mouse are biologically more distant from one another than human and chimpanzee or human and gorilla (Furano, A. V., Hayward, B. E., Chevret, P., Catzeflis, F., and Usdin, K. 1994. Amplification of the Ancient Murine Lx Family of Long Interspersed Repeated DNA Occurred During the Murine Radiation. *J Mol Evol* **38,** 18-27; Takahata, N., and Satta, Y. 1997. Evolution of the Primate Lineage Leading to Modern Humans: Phylogenetic and Demographic Inferences from DNA Sequences. *Proc Natl Acad Sci U S A* **94,** 4811-5). Furthermore, sheep and goats are biologically more distant from one another than human and chimpanzee (Randi, E., Fusco, G., Lorenzini, R., Toso, S., and Tosi, G. 1991. Allozyme Divergence and Phylogenetic Relationships Among Capra, Ovis and Rupicapra (Artyodactyla, Bovidae). *Heredity* **67,** 281-6. Nonetheless, these species give rise to viable chimeric animals (Meinecke-Tillmann, S., and Meinecke, B. 1984. *Nature* **307,** 637-8; Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. 1984. *Nature* **307,** 634-6).

Although Applicant believes its claims as originally drafted contain patentable subject matter, Applicant has amended the claims to chimeric embryos containing human and non-human primate cells.

With regard to chimeras of three or more species, the Examiner responded "Applicants state that the existing knowledge is that three cell types would cooperate with one another in building embryonic tissues and organs. However, this opinion is merely speculative." However, several papers in the literature indicate that chimeras can be constructed from the blastomeres of more than two individuals. For example, Markert and Petters (Markert, C. L., and Petters, R. M. (1978). Manufactured Hexaparental Mice Show that Adults are Derived from Three Embryonic Cells. *Science* **202,** 56-8) reported:

> Two female chimeric mice have been produced by aggregates of three genetically marked eight-cell embryos. All three embryonic genotypes are clearly expressed in the pigment pattern of the adults. These hexaparental mice together with their littermates demonstrate that, in the 64-celled blastocyst, at least three cells, and probably only three, are the source of all adult tissues.

These investigators subsequently reported (Petters, R. M., and Markert, C. L. 1980. Production and Reproductive Performance of Hexaparental and Octaparental mice. *J Hered* **71,** 70-4):

> The production of hexaparental and octaparental mice following embryo aggregation is reported. These mice were progeny-tested to determine which cell components were contributing to gamete formation. One chimera (No. 15) was shown to be producing eggs from all three cell strains--white, yellow, and black. The other chimeras tested were not forming germ cells from all of the cell strains involved. The use of these animals for obtaining a minimum estimate of the number of cells giving rise to the embryo proper is discussed. The suggestion is made that multi-embryo aggregates may actually be developmentally different from normal sized embryos and this may result in modified mechanisms of cell recruitment.

Thus cells from three different embryos can cooperate with one another to form tissues and organs, and indeed autonomous animals. It is reasonable to expect that any species whose blastomeres can cooperate to form an embryo (e.g., sheep and goat) would give rise to an informative developmental result (as in Petters and Markert, 1980) if cells from three individuals, even from three different such species, were used.

With regard to issued patents claiming "unpredictable" subject matter, for example, the oncomouse patent U.S. Patent No. 4,736,866, applicants claimed any nonhuman animal with a transgenic oncogene. There is no *a priori* way of knowing that such an animal would develop or survive as much as a day after birth. Oncogenes comprise at least 60 genes with a wide variety of biological functions. Some were even not discovered at the time the oncomouse application was

filed, but as far as Applicant understands were prospectively included in their claims. In the paper of Thomson et al., 1998, Thomson, J. A., Itskovitz-Eldor, J., Shapiro, S. S., Waknitz, M. A., Swiergiel, J. J., Marshall, V. S., and Jones, J. M. 1998. Embryonic Stem Cell Lines Derived from Human Blastocysts. *Science* 282, 1145-7 describing ES cell lines, the following statement appears:

> The human ES cell lines were derived by the selection and expansion of individual colonies of a uniform, undifferentiated morphology, but none of the ES cell lines was derived by the clonal expansion of a single cell. The uniform undifferentiated morphology that is shared by human ES and nonhuman primate ES cells and the consistent expression by the human ES cell lines of cell surface markers that uniquely characterize primate ES and human EC cells make it extremely unlikely that a mixed population of precursor cells was expanded. However, because the cell lines were not cloned from a single cell, we cannot rule out the possibility that there is some variation in developmental potential among the undifferentiated cells, in spite of their homogeneous appearance.

The Examiner contends that the chimeric embryo cannot be used for the purposes stated in the application unless it reaches a certain stage of development. The Examiner contends that the chimeric embryo must survive long enough to ensure that the cells are cooperating, i.e., no longer functioning independently, but together in the formation of one entity. The Examiner contends also that it is not clear that distant species would pass this level of viability and even with closer related species, and it is unpredictable that primate cells would be viable to this point. Applicant maintains that chimeras exist that have been made using primate cells, other than the limited number of species that have been disclosed. Applicant submits that the chimeric embryo may be useful during any stage of development.

The quotations from Gardner and Johnson (1975), above, and Petters and Markert (1980), above, indicate that even abortive, incomplete, or abnormal development is illuminating concerning

fundamental mechanisms of development, one of the states uses of the invention. But in the case of chimeric embryos constructed from humans and chimpanzees or humans and gorillas, pairs whose members share more than 98 percent DNA homology, it is expected that development will proceed to birth if given the opportunity.

The Examiner contends that even the human-mouse chimera represents a wide diversity which would not likely be viable long enough to become a cooperative entity. Applicant provides evidence below that interspecies chimeras would develop to this "cooperative" stage. Applicant maintains that chimeric embryos containing human cells would remain viable long enough to become cooperative entities.

Early embryos are extremely resilient to experimental perturbations, including separation of blastomeres, which typically leads to multiple well-formed individuals, and aggregation of blastomeres, leading to well-formed chimeras with intraspecific combinations and certain or inter-specific combinations, and otherwise, as in the case of mouse-rat chimeras, informative partial development. Using the search term "chimeric embryo," 633 citations were identified in a Medline search, and "embryo chimera" identified 1121 citations. Most of these citations did not pertain to the aggregation or injection chimeras under present consideration, but they were inclusive of those. Combining either of these search terms with "nonviable" or "non-viable" led to no hits, although 1241 instances of "nonviable" alone and 567 instances of "non-viable" alone appear in the Medline database. Thus nonviability of the outcome would not be a result expected by someone skilled in the art with regard to attempts to produce chimeric embryos of any type.

The term "failed to develop" appears in the database 2368 times. When combined with the term "chimera" and "embryo" three citations were identified, only one of which relates to the kind of

embryo chimeras claimed in the present invention. Combining "failed to develop" with "chimeric' and "embryo" turns up one additional citation. The first of these citations is: Fassler, R., and Meyer, M. (1995). Consequences of Lack of Beta 1 Integrin Gene Expression in Mice. *Genes Dev* **9,** 1896-908, and the summary of this paper is as follows:

> beta 1 integrins are cell-surface receptors that mediate cell-cell and cell-matrix interactions. We have generated a null mutation in the gene for the beta 1 integrin subunit in mice and embryonic stem (ES) cells. Heterozygous mice are indistinguishable from normal littermates. Homozygous null embryos develop normally to the blastocyst stage, implant, and invade the uterine basement membrane but die shortly thereafter. Using beta 1 integrin-deficient ES cells we have established chimeric embryos and adult mice. Analysis of the chimeric embryos demonstrated the presence of beta 1 integrin-deficient cells in all germ layers indicating that beta 1-null cells can differentiate and migrate in a context of normal tissue. When evaluated at embryonic day 9.5 (E9.5), embryos with a beta 1-null cell contribution below 25% were developing normally, whereas embryos with a contribution above this threshold were distorted and showed abnormal morphogenesis. In adult chimeric mice beta 1 integrin-deficient cells failed to colonize liver and spleen but were found in all other tissues analyzed at levels from 2%-25%. Immunostaining of chimeric mice showed that in cardiac muscle, there were small, scattered patches of myocytes that were beta 1-null. In contrast, many myotubes showed some beta 1-null contribution as a result of fusion between wild-type and mutant myoblasts to form mixed myotubes. The adult chimeric brain contained beta 1-null cells in all regions analyzed. Also, tissues derived from the neural crest contained beta 1 integrin-deficient cells indicating that migration of neuronal cells as well as neural crest cells can occur in the absence of beta 1 integrins.

The second citation is: Voss, A. K., Thomas, T., and Gruss, P. (2000). Mice lacking HSP90beta fail to develop a placental labyrinth. *Development* **127,** 1-11, and its summary reads:

> The 90 kDa heat-shock proteins (HSP90s) play important roles during stress situations as general chaperones and under physiological conditions in the conformational activation of specific protein substrates. Vertebrates express two cytosolic HSP90s (HSP90alpha and HSP90beta) ubiquitously. We have mutated the Hsp90beta gene in murine embryonic stem cells and generated Hsp90beta mutant mice. Heterozygous animals were phenotypically normal. Interestingly, homozygous embryos developed normally until embryonic day 9.0/9.5. Then, although Hsp90beta is expressed ubiquitously, they exhibited phenotypic abnormalities restricted to the placenta. The mutant concepti failed to form a fetal placental labyrinth and died a day later. Fusion between the allantois and the chorionic plate occurred, allantoic blood vessels invaded the chorion, but then did not expand. Mutant trophoblast cells failed to differentiate into trilaminar labyrinthine trophoblast. Despite conspicuous similarities between HSP90alpha and HSP90beta at the molecular level, our data suggest that HSP90beta has a key role in placenta development that cannot be performed by the endogenous HSP90alpha alone. Analysis of chimeric concepti consisting of mutant embryos and tetraploid embryos or ES cells revealed that wild-type allantois was able to induce mutant trophoblast to differentiate. In contrast, trophoblast wild type at the Hsp90beta locus was unable to differentiate when in contact with mutant allantois. Therefore, the primary defect caused by the Hsp90beta mutation resided in the allantois. The allantois mesoderm is thought to induce trophoblast differentiation. Our results show that Hsp90beta is a necessary component of this induction process.

One may conclude that (i) lack of viability or failure to develop at all is not found when chimeric embryos are constructed; and (ii) even when one of the cell types constituting the chimera is highly compromised genetically, e.g., completely lacking beta-integrin as in Fassler and Meyer (1995), or completely lacking Hsp90beta in Voss et al. (2000), which in each case is inconsistent with full development in the homozygous state, informative partial or full development of the chimeras nonetheless occurs. The human-chimpanzee and human-gorilla chimeras of the present invention consist of cells from genetically uncompromised organisms that are biologically as closely related, or more closely related, than sheep and goats, which can sustain full development as

chimeras. It would therefore be expected by anyone skilled in the art that chimeras constructed from human and nonhuman primate blastomeres would be viable for a sufficient length of time to generate informative data.

The summaries of the papers of Fassler and Meyer (1995) and Voss et al. (2000) are given in full to illustrate the insight into development that can be gained even from chimeras that develop incompletely -- a major element of utility of the human-nonhuman chimeras of the present invention.

IV. The Claims Satisfy 35 U.S.C. § 112, Second Paragraph

Claims 1-55 are rejected under 35 USC § 112, Second Paragraph, as being indefinite. This rejection is respectfully traversed. Applicant respectfully submits that the above-outlined amended claims and following remarks obviate the grounds for the rejection. Reconsideration and withdrawal of the rejection are respectfully requested.

The Examiner states that Claims 1-55 are "indefinite" due to their recitation of the phrase "chimeric embryos", based on the assertion that one would not know when a viable chimeric embryo was formed.

By "viable embryo," Applicant is referring to a chimeric embryo which is alive (in the sense of respiration and not necessarily progressing full term) and capable of developing to the next or successive stage of development, as the term "viable embryo" is used in the developmental and reproductive biology literature (see Loi et al. (1999) and Behr et al. (1999), cited above, for examples). As discussed above, and as evidenced by the papers of Gardner and Johnson (1975) on mouse-rat chimeras, Fassler and Meyer (1995), and Voss et al. (2000), anyone of ordinary skill in the art would be capable of using routine techniques of microscopy and histology to determine when

aggregated cells become a viable embryo. The fact that not all "viable" embryos would give rise to animals is not grounds for rejection of the claimed invention.

The Examiner states that Claims 13-18 are "indefinite" due to the recitation of the phrase "developed from". "Developed from a chimeric embryo" has an unambiguous meaning in the scientific literature, which uses "develop" to refer to "embryonic development," or advancement to successive stage(s), and not to "reproduction." In the original application reference is made to cells being developed from a chimeric embryo. The sense in which "developed" was used was the general one of "to promote the growth of" (Webster's Ninth New Collegiate Dictionary), rather than the reproductive sense of developing according to an ontogeneic program. Even though Applicant believes the original claim language adequately describes the present invention, Applicant has amended the subject claims by replacing the term "developed" with the term "derived" to address the Examiner's concerns.

The Examiner states that Claim 10 remains indefinite because the phraseology is unclear as to what would be required for a cell line to be "generated" from a chimeric embryo. For clarity, Applicant has followed the Examiner's recommendation and amended Claim 10 to read, "A cell line **isolated** from a chimeric embryo . . ."

Cells of chimeras are known to differ in some respects from equivalent cells in non-chimeric animals, leading for example, to immunological tolerance of non-chimeric mothers to chimeras (Meinecke-Tillmann, S., and Meinecke, B. (1984) Experimental Chimaeras--Removal of Reproductive Barrier Between Sheep and Goat. *Nature* **307,** 637-8; Fehilly, C. B., Willadsen, S. M., and Tucker, E. M. (1984). Interspecific Chimerism Between Sheep and Goat. *Nature* **307,** 634-6), of chimeras to grafted cells of the originating species (Gustafson, R. A., Anderson, G. B.,

BonDurant, R. H., and Mahi-Brown, C. (1993). Tolerance of Sheep-Goat Chimeras to their Component Cells. *J. Reprod. Immunol.* **23,** 155-68), and of reproductive efficacy of germ cells. The cell lines of the invention would, for example, permit the study of such immunological and reproductive properties at the cellular level.

V. Conclusion

In view of the foregoing amendments and remarks, Applicant respectfully submits that the claims define statutory subject matter that is patentable over the art of record and the application is in condition for allowance. Should the Examiner believe anything further is desirable to place the application in better condition for allowance, the Examiner is invited to contact Applicant's undersigned attorney at the telephone number listed below.

Respectfully Submitted,

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